

Product datasheet

Anti-p53 antibody [Y5] ab32049

KO **VALIDATED**

Recombinant

RabMAb[®]

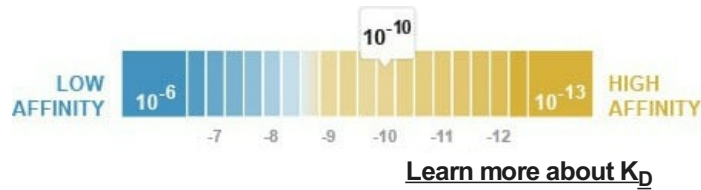
★★★★☆ **5 Abreviews** **54 References** **21 Images**

Overview

Product name	Anti-p53 antibody [Y5]
Description	Rabbit monoclonal [Y5] to p53
Host species	Rabbit
Specificity	<p>This antibody clone recognises both wild-type and mutant forms of p53 in human samples. It is not designed to recognise any specific p53 mutation.</p> <p>We have confirmed this experimentally and have been able to detect p53 in different cell lines using various applications and treatments.</p> <p>Important note: p53 expression levels vary greatly between cell lines. It has been reported that p53 mutations render the protein more stable, hence mutated cell lines often express higher levels of the p53 protein compared to wild-type cell lines. For low expressing wild type cell lines, p53 expression can be increased with cell treatments such as camptothecin or irinotecan.</p>
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	<p>Wild type p53: A549, HEK293, HepG2, MCF7, U-87 MG. Mutant p53: A431 (R273H), DU 145 (P223L and V274F), HAP1 (S215G), Jurkat (R196*), MDA-MB-435 (G266E), Raji (R213Q and Y234H), Ramos (I254N), SK-BR-3 (R175H), T-47D (L194F). Cell lines expressing the highest levels of p53 without induction are HEK293 (WT p53), A431 and HAP1 (mutant p53). Negative cell line: Saos-2. IHC-P controls: Bladder, Skin Cancer, Glioma, Gastric adenocarcinoma, Human breast and lung carcinoma tissue, Human colon adenocarcinoma.</p>
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Dissociation constant (K_D)	$K_D = 2.02 \times 10^{-10}$ M



Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y5
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32049 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF	★☆☆☆☆ (1)	1/100.
WB	★★★★★ (4)	1/1000 - 1/5000. Detects a band of approximately 53 kDa (predicted molecular weight: 44 kDa).
IHC-P		1/50. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/20.

Target

Function	Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. Implicated in Notch signaling cross-over. Isoform 2 enhances the transactivation activity of isoform 1 from some but not all TP53-inducible promoters. Isoform 4
----------	--

suppresses transactivation activity and impairs growth suppression mediated by isoform 1. Isoform 7 inhibits isoform 1-mediated apoptosis.

Tissue specificity

Ubiquitous. Isoforms are expressed in a wide range of normal tissues but in a tissue-dependent manner. Isoform 2 is expressed in most normal tissues but is not detected in brain, lung, prostate, muscle, fetal brain, spinal cord and fetal liver. Isoform 3 is expressed in most normal tissues but is not detected in lung, spleen, testis, fetal brain, spinal cord and fetal liver. Isoform 7 is expressed in most normal tissues but is not detected in prostate, uterus, skeletal muscle and breast. Isoform 8 is detected only in colon, bone marrow, testis, fetal brain and intestine. Isoform 9 is expressed in most normal tissues but is not detected in brain, heart, lung, fetal liver, salivary gland, breast or intestine.

Involvement in disease

Note=TP53 is found in increased amounts in a wide variety of transformed cells. TP53 is frequently mutated or inactivated in about 60% of cancers. TP53 defects are found in Barrett metaplasia a condition in which the normally stratified squamous epithelium of the lower esophagus is replaced by a metaplastic columnar epithelium. The condition develops as a complication in approximately 10% of patients with chronic gastroesophageal reflux disease and predisposes to the development of esophageal adenocarcinoma. Defects in TP53 are a cause of esophageal cancer (ESCR) [MIM:133239]. Defects in TP53 are a cause of Li-Fraumeni syndrome (LFS) [MIM:151623]. LFS is an autosomal dominant familial cancer syndrome that in its classic form is defined by the existence of a proband affected by a sarcoma before 45 years with a first degree relative affected by any tumor before 45 years and another first degree relative with any tumor before 45 years or a sarcoma at any age. Other clinical definitions for LFS have been proposed (PubMed:8118819 and PubMed:8718514) and called Li-Fraumeni like syndrome (LFL). In these families affected relatives develop a diverse set of malignancies at unusually early ages. Four types of cancers account for 80% of tumors occurring in TP53 germline mutation carriers: breast cancers, soft tissue and bone sarcomas, brain tumors (astrocytomas) and adrenocortical carcinomas. Less frequent tumors include choroid plexus carcinoma or papilloma before the age of 15, rhabdomyosarcoma before the age of 5, leukemia, Wilms tumor, malignant phyllodes tumor, colorectal and gastric cancers. Defects in TP53 are involved in head and neck squamous cell carcinomas (HNSCC) [MIM:275355]; also known as squamous cell carcinoma of the head and neck. Defects in TP53 are a cause of lung cancer (LNCr) [MIM:211980]. Defects in TP53 are a cause of choroid plexus papilloma (CPLPA) [MIM:260500]. Choroid plexus papilloma is a slow-growing benign tumor of the choroid plexus that often invades the leptomeninges. In children it is usually in a lateral ventricle but in adults it is more often in the fourth ventricle. Hydrocephalus is common, either from obstruction or from tumor secretion of cerebrospinal fluid. If it undergoes malignant transformation it is called a choroid plexus carcinoma. Primary choroid plexus tumors are rare and usually occur in early childhood. Defects in TP53 are a cause of adrenocortical carcinoma (ADCC) [MIM:202300]. ADCC is a rare childhood tumor of the adrenal cortex. It occurs with increased frequency in patients with the Beckwith-Wiedemann syndrome and is a component tumor in Li-Fraumeni syndrome.

Sequence similarities

Belongs to the p53 family.

Domain

The nuclear export signal acts as a transcriptional repression domain. The TAD1 and TAD2 motifs (residues 17 to 25 and 48 to 56) correspond both to 9aaTAD motifs which are transactivation domains present in a large number of yeast and animal transcription factors.

Post-translational modifications

Acetylated. Acetylation of Lys-382 by CREBBP enhances transcriptional activity. Deacetylation of Lys-382 by SIRT1 impairs its ability to induce proapoptotic program and modulate cell senescence.

Phosphorylation on Ser residues mediates transcriptional activation. Phosphorylated by HIPK1 (By similarity). Phosphorylation at Ser-9 by HIPK4 increases repression activity on BIRC5 promoter. Phosphorylated on Thr-18 by VRK1. Phosphorylated on Ser-20 by CHEK2 in response

to DNA damage, which prevents ubiquitination by MDM2. Phosphorylated on Thr-55 by TAF1, which promotes MDM2-mediated degradation. Phosphorylated on Ser-46 by HIPK2 upon UV irradiation. Phosphorylation on Ser-46 is required for acetylation by CREBBP. Phosphorylated on Ser-392 following UV but not gamma irradiation. Phosphorylated upon DNA damage, probably by ATM or ATR. Phosphorylated on Ser-15 upon ultraviolet irradiation; which is enhanced by interaction with BANP.

Dephosphorylated by PP2A-PPP2R5C holoenzyme at Thr-55. SV40 small T antigen inhibits the dephosphorylation by the AC form of PP2A.

May be O-glycosylated in the C-terminal basic region. Studied in EB-1 cell line.

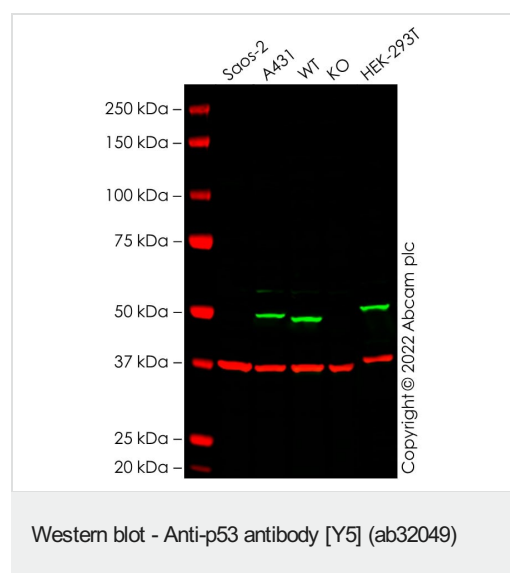
Ubiquitinated by MDM2 and SYVN1, which leads to proteasomal degradation. Ubiquitinated by RFWD3, which works in cooperation with MDM2 and may catalyze the formation of short polyubiquitin chains on p53/TP53 that are not targeted to the proteasome. Ubiquitinated by MKRN1 at Lys-291 and Lys-292, which leads to proteasomal degradation. Deubiquitinated by USP10, leading to its stabilization. Ubiquitinated by TRIM24, which leads to proteasomal degradation. Ubiquitination by TOPORS induces degradation. Deubiquitination by USP7, leading to stabilization. Isoform 4 is monoubiquitinated in an MDM2-independent manner.

Monomethylated at Lys-372 by SETD7, leading to stabilization and increased transcriptional activation. Monomethylated at Lys-370 by SMYD2, leading to decreased DNA-binding activity and subsequent transcriptional regulation activity. Lys-372 monomethylation prevents interaction with SMYD2 and subsequent monomethylation at Lys-370. Dimethylated at Lys-373 by EHMT1 and EHMT2. Monomethylated at Lys-382 by SETD8, promoting interaction with L3MBTL1 and leading to repress transcriptional activity. Demethylation of dimethylated Lys-370 by KDM1A prevents interaction with TP53BP1 and represses TP53-mediated transcriptional activation. Sumoylated by SUMO1.

Cellular localization

Cytoplasm; Cytoplasm. Nucleus. Nucleus > PML body. Endoplasmic reticulum. Interaction with BANP promotes nuclear localization. Recruited into PML bodies together with CHEK2; Nucleus. Cytoplasm. Localized in both nucleus and cytoplasm in most cells. In some cells, forms foci in the nucleus that are different from nucleoli; Nucleus. Cytoplasm. Localized in the nucleus in most cells but found in the cytoplasm in some cells; Nucleus. Cytoplasm. Localized mainly in the nucleus with minor staining in the cytoplasm; Nucleus. Cytoplasm. Predominantly nuclear but localizes to the cytoplasm when expressed with isoform 4 and Nucleus. Cytoplasm. Predominantly nuclear but translocates to the cytoplasm following cell stress.

Images



All lanes : Anti-p53 antibody [Y5] (ab32049) at 1/1000 dilution

Lane 1 : Saos-2 cell lysate

Lane 2 : A431 cell lysate

Lane 3 : Wild-type HAP1 cell lysate

Lane 4 : TP53 knockout HAP1 cell lysate

Lane 5 : HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

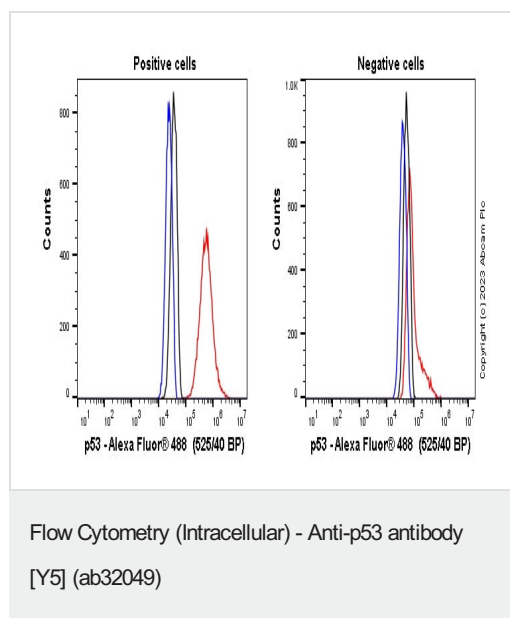
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

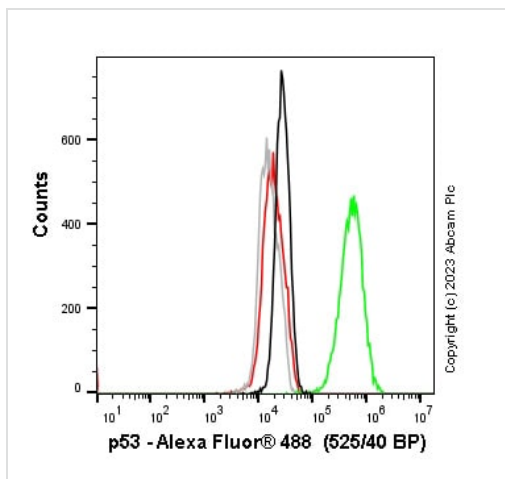
Predicted band size: 44 kDa

Observed band size: 50 kDa

False colour image of Western blot: Anti-p53 antibody [Y5] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] ([ab8245](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32049 was shown to bind specifically to p53. A band was observed at 50 kDa in wild-type Saos-2 cell lysates with no signal observed at this size in tp53 knockout cell line. To generate this image, wild-type and tp53 knockout Saos-2 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween[®] 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

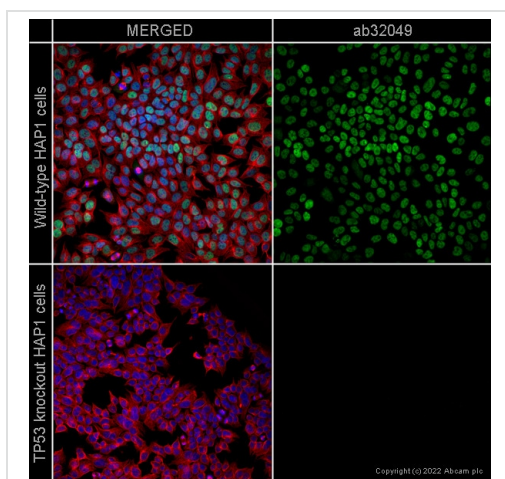


Flow cytometry overlay histogram showing wild-type p53 in Hek-293 positive cells (left) and MCF7 negative cells (right) stained with ab32049 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab32049) (1×10^6 cells in 100 μ l at 0.008 μ g/ml (1/13250)) for 30 min at 22°C. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed ([ab150081](#)) was incubated at 1/4000 for 30 min at 22°C. Isotype control antibody (black line) Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control ([ab172730](#)) was used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control. Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. This antibody gave a positive signal in Hek-293 fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Flow Cytometry (Intracellular) - Anti-p53 antibody
[Y5] (ab32049)

Flow cytometry overlay histogram showing p53 in wild-type HAP1 (green line) and TP53 knockout HAP1 (red line) cells stained with ab32049. The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab32049) (1×10^6 cells in 100 μ l at 0.2 μ g/ml (1/440)) for 30min at 22°C. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150081**) was incubated at 1/4000 for 30min at 22°C. Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) was used at the same concentration and conditions as the primary antibody (wild-type HAP1 - black line, TP53 knockout HAP1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. This antibody gave a positive signal in HAP1 Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



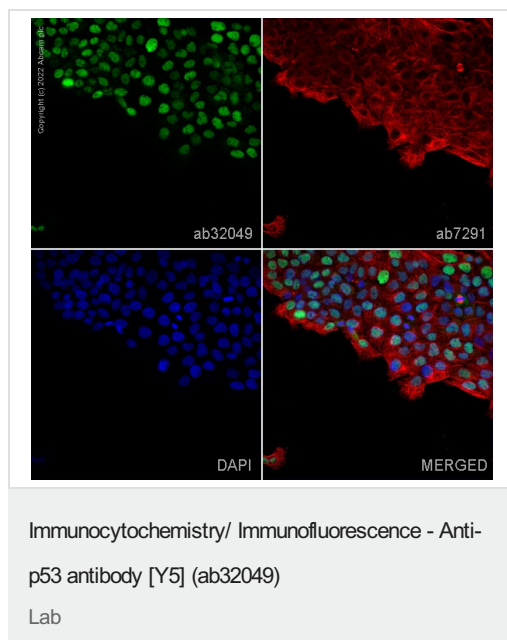
Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [Y5] (ab32049)

Lab

ab32049 staining p53 in wild-type Hap1 cells (top panel) and p53 knockout Hap1 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at °C with ab32049 at 0.2 μ g/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

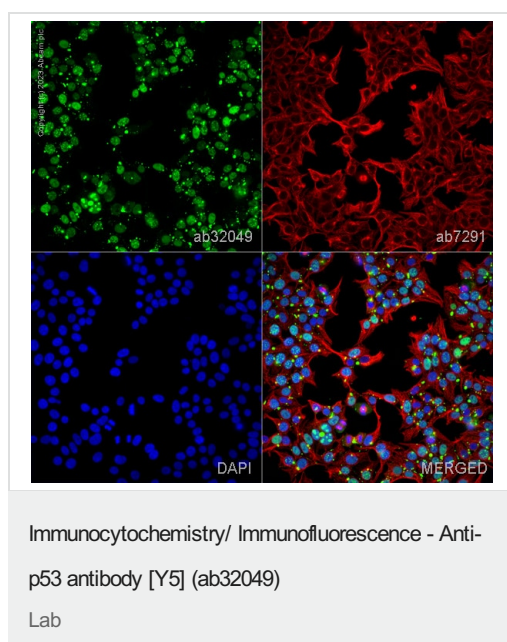
Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



ab32049 staining p53 in A431 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab32049 at 0.2µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

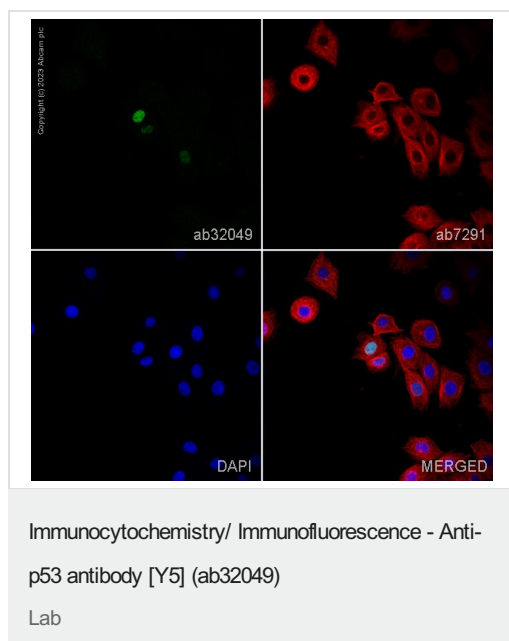
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



ab32049 staining wild-type p53 in Hek293 cells (a high expressing cell line). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab32049 at 0.2µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

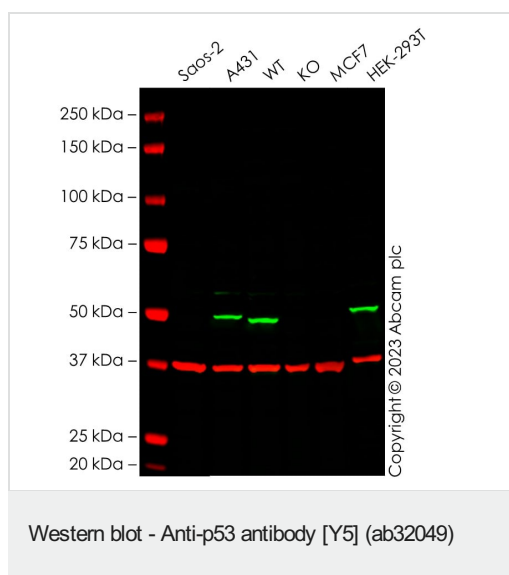
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



ab32049 staining wild-type p53 in MCF7 cells (a low expressing cell line). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab32049 at 0.2µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



All lanes : Anti-p53 antibody [Y5] (ab32049) at 1/1000 dilution

Lane 1 : Saos-2 cell lysate

Lane 2 : A431 cell lysate

Lane 3 : Wild-type HAP1 cell lysate

Lane 4 : TP53 knockout HAP1 cell lysate

Lane 5 : MCF7 cell lysate

Lane 6 : HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

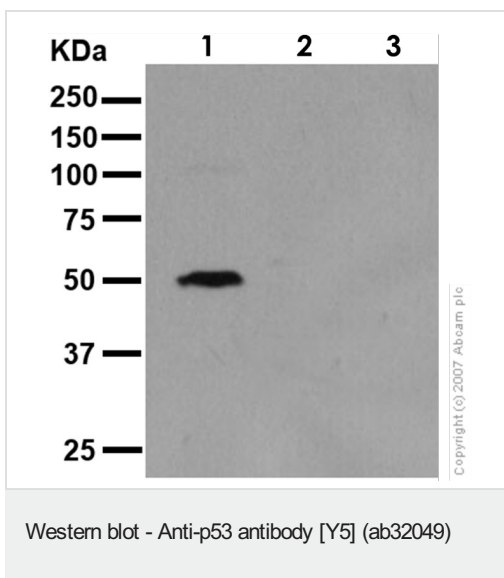
Performed under reducing conditions.

Predicted band size: 44 kDa

Observed band size: 50 kDa

False colour image of Western blot: Anti-p53 antibody [Y5] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32049 was shown to bind specifically to

p53. A band was observed at 50 kDa in wild-type HAP1 cell lysate with no signal observed at this size in tp53 knockout cell line. To generate this image, wild-type and tp53 knockout HAP1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween[®] 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.



All lanes : Anti-p53 antibody [Y5] (ab32049) at 1/10000 dilution

Lane 1 : A431 (Human epidermoid carcinoma epithelial cell), Whole cell lysate

Lane 2 : A549 (Human lung carcinoma epithelial cell), Whole cell lysate

Lane 3 : MCF-7 (Human breast adenocarcinoma epithelial cell), Whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated ([ab97051](#)) at 1/20000 dilution

Predicted band size: 44 kDa

Exposure time: 180 seconds

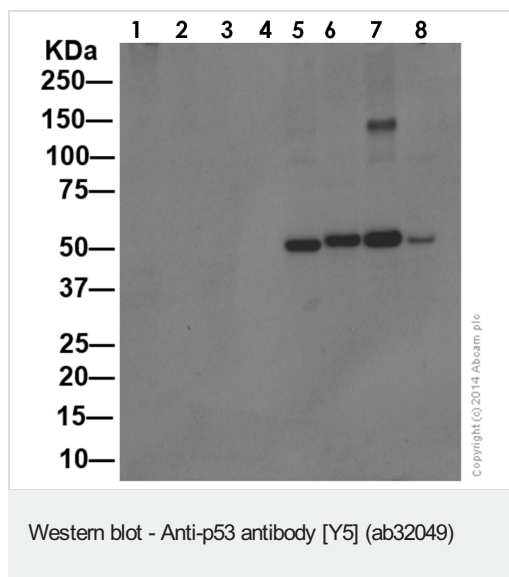
Blocking/Diluting buffer and concentration: 5% NFDM/TBST

Lane1: Mutant p53 cell lines

Lane2-3: Wildtype p53 cell lines

Exposure time: 180 seconds

Observed MW: 50KDa



All lanes : Anti-p53 antibody [Y5] (ab32049) at 1/500 dilution

Lane 1 : HepG2 (Human hepatocellular carcinoma epithelial cell), Whole cell lysate

Lane 2 : A549 (Human lung carcinoma epithelial cell), Whole cell lysate

Lane 3 : MCF-7 (Human breast adenocarcinoma epithelial cell), Whole cell lysate

Lane 4 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell), Whole cell lysate

Lane 5 : MDA-MB-435 (Human mammary gland ductal carcinoma melanocyte), Whole cell lysate

Lane 6 : T-47D (Human ductal breast epithelial tumor epithelial cell), Whole cell lysate

Lane 7 : Raji (Human Burkitt's lymphoma B lymphocyte), Whole cell lysate

Lane 8 : Ramos (Human Burkitt's lymphoma B lymphocyte), Whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated ([ab97051](#)) at 1/20000 dilution

Predicted band size: 44 kDa

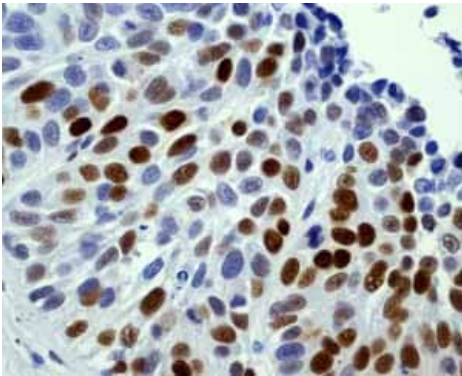
Exposure time: 60 seconds

Blocking/Diluting Buffer and concentration: 5% NFDM/TBST

Lane 1-4: Wildtype p53 cell lines

Lane 5-8: Mutant p53 cell lines

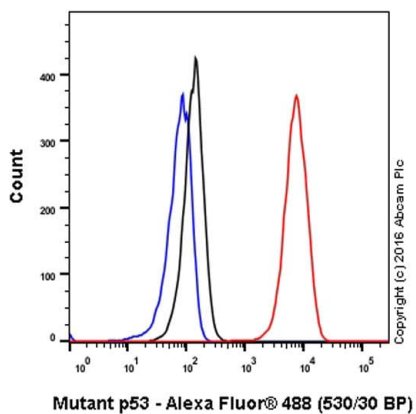
Observed MW: 50 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [Y5] (ab32049)

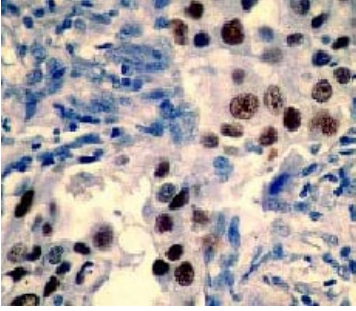
ab32049 showing positive staining in Urinary bladder carcinoma tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-p53 antibody [Y5] (ab32049)

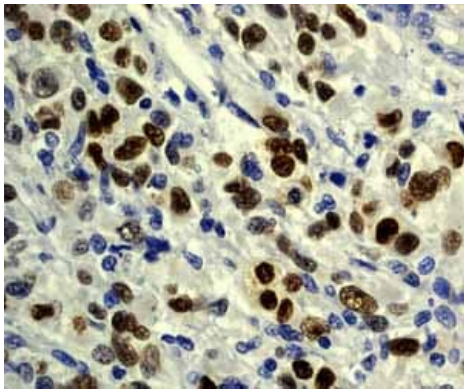
Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labeling p53 with unpurified ab32049 at 1/20 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [Y5] (ab32049)

Immunohistochemistry (Paraffin-embedded sections) using ab32049 at a dilution of 1/50 and human skin cancer

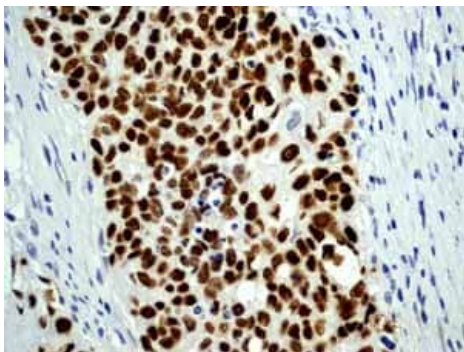
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [Y5] (ab32049)

ab32049 showing positive staining in Glioma tissue.

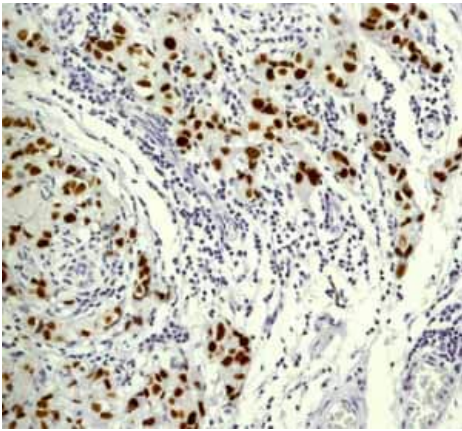
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [Y5] (ab32049)

ab32049 showing positive staining in Gastric adenocarcinoma tissue.

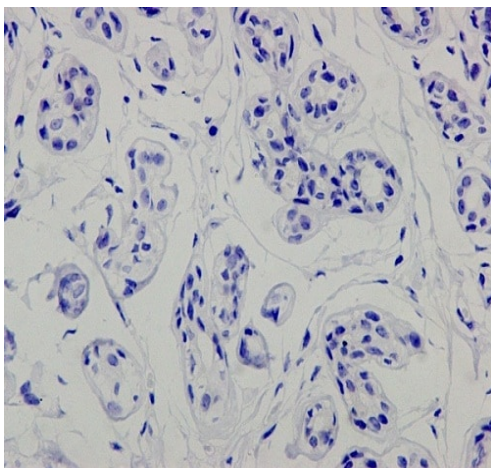
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [Y5] (ab32049)

ab32049 showing positive staining in Breast carcinoma tissue.

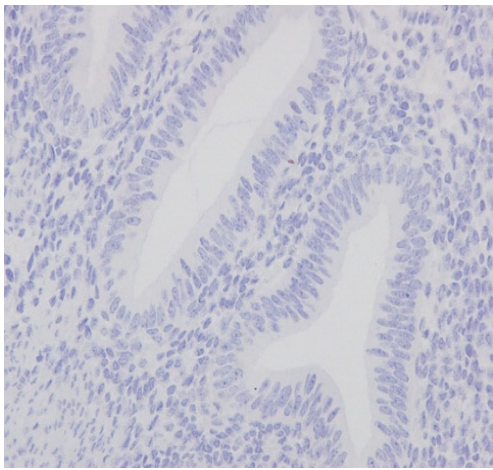
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [Y5] (ab32049)

Immunohistochemical analysis of paraffin embedded normal Human breast tissue (negative control) labeling p53 with ab32049.

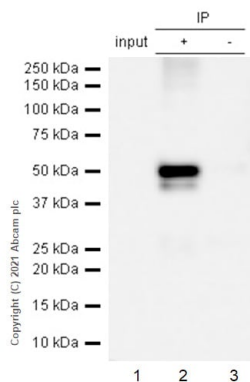
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [Y5] (ab32049)

Immunohistochemical analysis of paraffin embedded normal Human uterus tissue (negative control) labeling p53 with ab32049.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-p53 antibody [Y5] (ab32049)

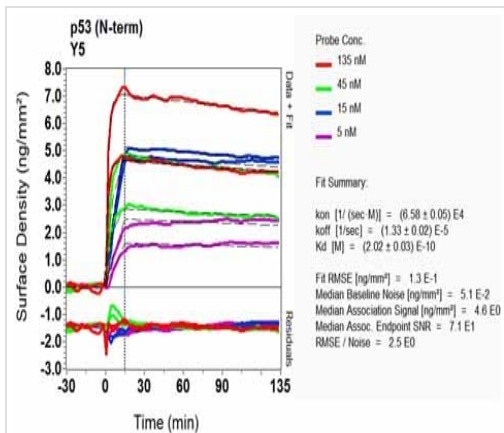
p53 was immunoprecipitated from 0.35 mg A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate 10 µg with ab32049 at 1/100 dilution (2µg) . VeriBlot for IP Detection Reagent (HRP)([ab131366](#)) was used at 1/5000 dilution.

Lane 1: A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate 10 µg

Lane 2: ab32049 IP in A431 whole cell lysate

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab32049 in A431 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



OI-RD Scanning - Anti-p53 antibody [Y5] (ab32049)

Equilibrium disassociation constant (K_D)

Learn more about K_D

[Click here to learn more about \$K_D\$](#)

Why choose a recombinant antibody?



Research with confidence
 Consistent and reproducible results



Long-term and scalable supply
 Recombinant technology



Success from the first experiment
 Confirmed specificity



Ethical standards compliant
 Animal-free production

Anti-p53 antibody [Y5] (ab32049)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise,

please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors